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Inhibitory activity of a betacyanin formulation from red pitahaya (*Hylocereus polyrhizus*) and red spinach (*Amaranthus dubius*) against polymicrobial biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Staphylococcus aureus (Gram-positive bacteria) and *Pseudomonas aeruginosa* (Gram-negative bacteria) are common biofilm forming bacterial species in many resistant infections. This study investigated the effect of a combination of betacyanin fractions from red pitahaya and red spinach on inhibition of polymicrobial biofilm formation by *S. aureus* and *P. aeruginosa.* Betacyanin fractions were obtained from the pulp of red pitahaya and the leaves of red spinach. Screening of formulations containing various concentrations of the betacyanin fractions showed that the formulation combining 0.625 mg mL^{-1} of each betacyanin fraction inhibited 30.3–45.6% of biofilm formation by five *S. aureus* strains and 32.5–49.8% of biofilm formation by four *P. aeruginosa* strains on polystyrene surfaces. This formulation was 7–9% more effective in inhibiting polymicrobial biofilm of *S. aureus* ATCC 6538P and *P. aeruginosa* ATCC 27853 on polystyrene surfaces compared to using a single fraction. The betacyanin fraction formulation also significantly inhibited polymicrobial biofilms (62.8–78.0%) and reduced bacterial attachment (0.98–1.30 log CFU cm^{-2}) on various other polymer surfaces (polypropylene, polyethylene, polyvinyl chloride and silicone rubber). The betacyanin fraction formulation improved anti-biofilm activity against co-culture of *S. aureus* and *P. aeruginosa* biofilm as compared to individual fractions.

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